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THE UTILITY OF CYTOKERATINS IMMUNOHISTOCHEMISTRY IN THE DISTINCTION OF GASTRO-ESOPHAGEAL ADENOCARCINOMAS

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ABSTRACT

The gastro-esophageal junction is regularly exposed to concentrated gastric acid and to a variety of nitrosating species, noxious agents that may contribute to carcinogenesis in this region. Adenocarcinomas of the gastric cardia and distal oesophagus are among the most deadly of all gastro-intestinal malignancies, with 5-year mortality rates exceeding 80%. The incidence of both adenocarcinomas has been increasing rapidly for more than two decades in the Western world.

These gastro-esophageal junction carcinomas arise from intestinal metaplasia foci that develop either in the distal esophagus (Barrett's esophagus) or in the gastric cardia (cardiac intestinal metaplasia).

The intestinal metaplasia of the esophagus or of the cardia cannot be accurately differentiated on the basis of routine histology alone. Recent immunohistochemical studies have reported a potential role for cytokeratins 7 (CK7) and 20 (CK20) in differentiating the two.

In the present study we investigated the role of CK7/20 immunoreactivity combined with clinical characteristics and endoscopic appearances to distinguish of adenocarcinomas of the distal esophagus and gastric cardia.

KEYWORDS*: cytokeratins 7 and 20; Barrett's esophagus; cardiac intestinal metaplasia; distal esophageal adenocarcinoma; cardiac adenocarcinoma.*

1.Introduction

Adenocarcinomas of the distal esophagus and gastric cardia are two tumors of the gastro-esophageal junction. This junction is the line at which the esophagus ends and the stomach begins. Adenocarcinomas of the gastro-esophageal junction are malignancies that cross the line, so that part of the tumor is in the stomach and part is in the esophagus. There are no clear and universally accepted anatomic landmarks that delimit the distal esophagus and the gastric cardia, and investigations on adenocarcinomas of the junction have been hampered by difficulties in ascertaining whether these tumors are esophageal or gastric in origin [1,2]. Most studies on gastro-esophageal junction cancers have categorized the

tumors as "cardiac" or "esophageal" depending on the location of the tumor's epicenter. Whereas tumors may not grow symmetrically, the location of the tumor epicenter may not accurately identify the origin of a tumor at the junction [3].

Both tumors have a rising incidence, diagnosed frecvently in the sixth decade of life in white meales with obesity, are lethal diseases with an overall 5 year survival of only 10-20% [4]. Surgical cure rates (subtotal esophagectomy and proximal gastrectomy) are compromised by the fact that most patients are diagnosed at the late stage of disease of the delayed onset of symptoms.

The etiology of esophageal adenocarcinoma is related to gastro-esophageal reflux disease and the development of Barrett's esophagus, a pre-neoplasic condition which is diagnosed endoscopically with an histological confirmation, an incomplete intestinal metaplasia. The medical societies have recommended endoscopic cancer surveillance routinely for pacients with Barrett's metaplasia. The etiology of adenocarcinoma of the gastric cardia is less well understood, cardiac intestinal metaplasia is probably related to gastro-esophageal reflux disease and gastric *Helicobacter pylori* infection.

The presence of intestinal metaplasia at the gastric cardia isn't a strong predictor for the development of cardiac carcinoma and isn't recommended endoscopic cancer surveillance [5] Earlier detection of the metapasia-dysplasia-esophageal adenocarcinoma and distinction between short-segment Barrett's esophageal and cardiac intestinal metaplasia are essential for the aplication of the prophylaxis of malignant transformation.

We chosed the immunohistochemical study for cytokeratin 7 and cytokeratin 20 with "CK 7/20 staining pattern" [6,7,8] for evidence the distinction of Barrett's metaplasia from cardiac metaplasia and the potential of utilization in the early detection of adenocarcinoma.

2. Materials and methods

Patients

Thirty-three gastro-esophageal junction resection specimens of patients with metaplasia and adenocarcinomas were retrieved from the archives of the pathological departament of No. 1 Emergency Hospital of Craiova. All the resection specimens acquired with consent, belong to the patients underwent a surgical resection or biopsy to the gastro-esophageal junction in the period 1996-2005.

The patients included in this study presented symptoms of gastro-esophageal reflux disease which included: heartburn and regurgitation at least twice within 1 week and persisted at least six months, all the symptoms could be relieved significantly with H2 receptor blocker or proton pump inhibitor, odynophagia with progresiv dysphagia addition decrease weight, occult haemorrhages (seldom).

Endoscopy and biopsy protocol

Endoscopic dates included macroscopic changes to the gastro-esophageal junction: a red velvety mucosa it can extend either circumferentially or as one or several tongues, polipoid lesions, ulcerations, presence or absence of hiatus hernia.

The patients were classified into four groups acording to histological and endoscopic data:

Group I: eight patients with Barrett's metaplasia, wich was defined endoscopically as a red, velvet-like columnar mucosa in distal esophagus,

Group II: five patients with cardiac intestinal metaplasia, wich was defined endoscopically as a red, velvet- like mucosa were obtained within 2 cm below gastro-esophageal junction when this junction coincided precisely with squamo-columnar junction,

Group III: nine patients with distal esophageal adenocarcinoma, which was defined as a tumor predominant localization in the distal 8 cm of the oesophagus, including the intra-abdominal

oesophagus and association with Barrett's intestinal metaplasia,

Group IV: eleven patients with gastric cardia adenocarcinoma, which was defined as a tumor with predominant localization below the esophago-gastric junction and association with cardiac intestinal metaplasia.

For each resection specimen a sample of tumor tissue and normal tissue was available in paraffin –embedded slides.

The biopsy protocols included four quadrant biopsies at 2 cm levels in distal esophagus above and below the esophago-gastric junction and sample of normal tissue. Specimens were fixed in 10% buffered formalin, embedded in paraffin wax and carefully microdisected (5-µm slides). All sections were stained with hematoxylin-eosin (H&E) and mounted on glass slides.

Giemsa staining

Giemsa staining was used to assess *Helicobacter pylori* infection.

Immunohistochemistry for cytokeratins CK 7 and CK 20

We performed immunostaining on formalin –fixed, paraffin embedded tissue sections using the immuno-enzyme polymer method name LSAB/HRP (Universal DAKO Labbeled Streptavidin Biotin 2 System Horseradish Peroxidase). Five-micrometer-thick serial sections were cut from each paraffin-embedded block.

The sections were deparaffinized in xylene and rehydrated through graded concentrations of alcohol slides were incubated in 3% hydrogen peroxide for 20 min to block nonspecific background staining due to endogenous peroxidase.

Antigen retrieval was performed by using 10 mM citrate buffer, ph 6.0 for 10-20 min in microwave. Using the standard streptavidin-biotin peroxidase complex method were performed on all slides, were incubated for 30 minutes in secondary antibody solution. Diaminobenzidine (DAB) was used as a chromogen and developed a brown precipitate, very fine, localizing the antigen searched in the cytoplasm. The slides were counterstained with Mayer's hematoxylin.

Negative controls were counterstained with the same tumor samples and staining methods by omitting the primary antibodies.

We utilized intent antibodies with optimal dilutions and retrieval (table 1).

Table 1. *Antibodies, optimal dilution, incubation times and retrieval using for immunohistochemistry*

Antibodies	Clone	Dilution	Unmasking of antigen Retrieval	Incubation times
Cytokeratin CK20	M7019 IgG2a k	1:50	3 cicli MW (750 W) in buffer citrat	30 min TA
Cytokeratin CK7	M7018 IgG1k	1:40	3 cicli MW (750 W) in buffer citrat	30 min TA

Evaluation of cytokeratin staining

In this study we investigated the distribution and intensity of cytoplasmic cytokeratins CK7 and CK20 staining in Barrett's metaplasia, cardiac intestinal metaplasia or neoplasic epithelium. Evaluation of cytokeratins (CK) staining was determined in a semiquantitative fashion, as described by Ormsby *et al*, (1999): Barrett CK7/CK20 pattern was characterised by strong diffuse CK7 and superficial CK20 immunostaining. Gastric CK7/CK20 pattern depended on the histochemical type of intestinal metaplasia: complete metaplasia was characterised by absent CK7 immunostaining and strong diffuse CK20 immunostaining, whereas incomplete metaplasia was characterised by weak patchy CK7 staining and moderate patchy CK20

immunostaining. All the patterns that did not belong to Barrett's or gastric patterns were excluded.

3. Results

The thirty-three resection specimens studious presents: Barrett's metaplasia (figure 1,2), cardial intestinal metaplasia, distal esophageal adenocarcinoma (figure 3,4) and gastric adenocarcinoma, with histologic different stage (table 2).

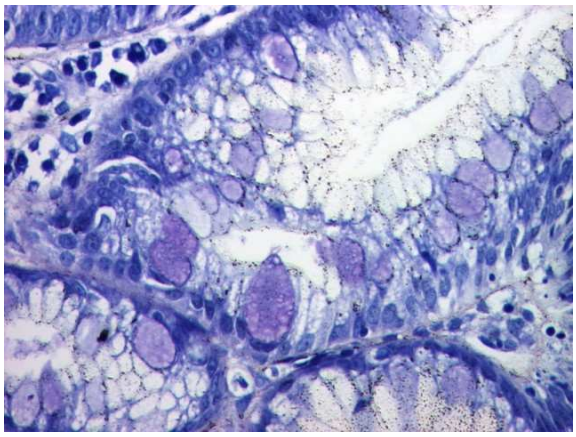

Figure 1. *Barrett's esophagus, (Giemsa staining, ob. 20x)*

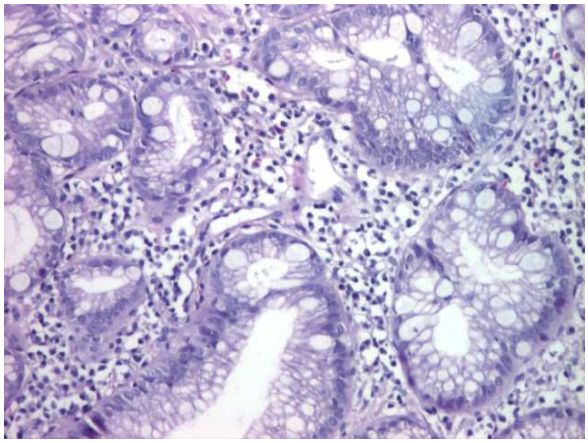

Figure 2. *Barrett's esophagus, (Hematoxylin-eosin staining, ob. 4x)*

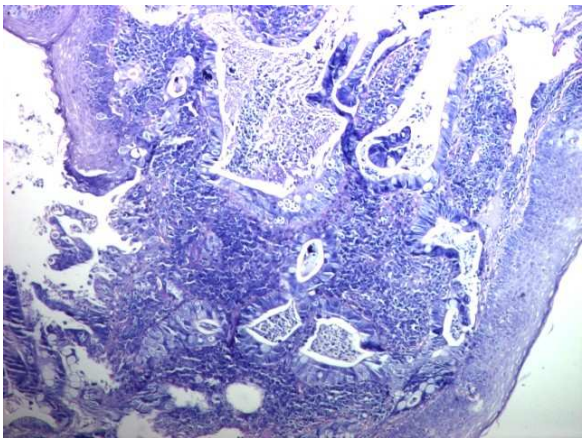

Figure 3. *Distal esophageal adenocarcinoma, (Giemsa staining, ob. 4x)*

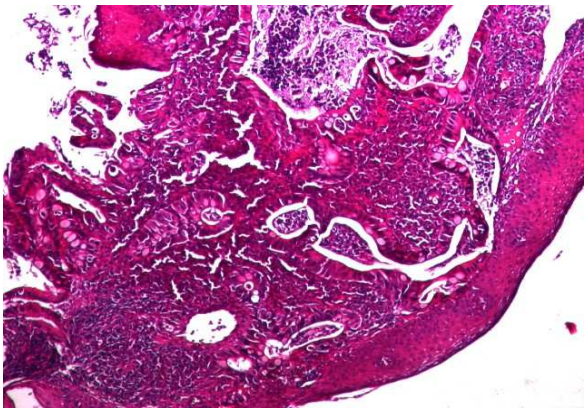

Figure 4. *Distal esophageal adenocarcinoma, (Hematoxylin-eosin staining, ob. 4x)*

Table 2: *Patients and tumor characteristics*

	Barrett's metaplasia n = 8	Cardial intestinal metaplasia n = 5	Distal esophageal adeno carcinoma n = 9	Dastric cardia adeno carcinoma n = 11
Sex F	2	0	1	3
M	6	5	8	8
Age	63.2	59,1	64,8	69,8
Range	38 – 86	39 – 79	55 – 82	58 – 86
Tumor stage: II A			0	1
II B			0	3
III			3	5
IV			6	2

The CK7/20 immunoreactivity patterns at different sites are shown in table 3.

Table 3. *CK7/20 immunoreactivity pattern*

Histological types	n	Barrett CK7/CK20 pattern	Gastric CK7/CK20 pattern
Barrett's metaplasia (group I)	8	5 (62,5%)	1 (12,5%)
Cardial intestinal metaplasia (group II)	5	1 (20%)	3 (60%)
Distal esophageal adenocarcinoma (group III)	9	6 (66,6%)	1 (11,1%)
Gastric cardia adenocarcinoma (group IV)	11	2 (18,1%)	7 (63,6)

The CK 7/20 immunoreactivity pattern in Barrett's metaplasia was mainly "Barrett's CK7/20 pattern" (62,5%), similar to distal esophageal adenocarcinoma (66,6%) (figure 5, 6), but there were still 12,5% and, respectively, 11,1% cases which showed gastric CK7/20 pattern.

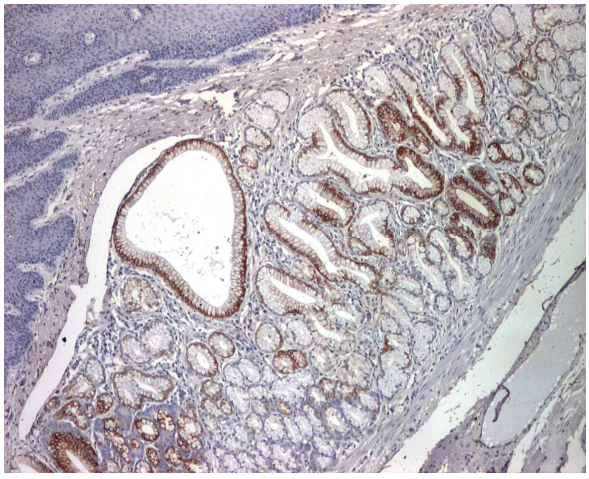

Figure 5. *Strong CK 7 immunostaining in esophageal adenocarcinoma, (ob.4x)*

On the contrary, the preponderant CK7/20 immunoreactivity pattern in cardiac intestinal metaplasia was "gastric CK7/20 pattern"(60%), similar to gastric cardia adenocarcinoma (63,6%) (figure 7, 8).

Cardiac intestinal metaplasia and gastric cardia adenocarcinoma were associated in 20% and, respectively, 18,1% cases with Barrett's pattern.

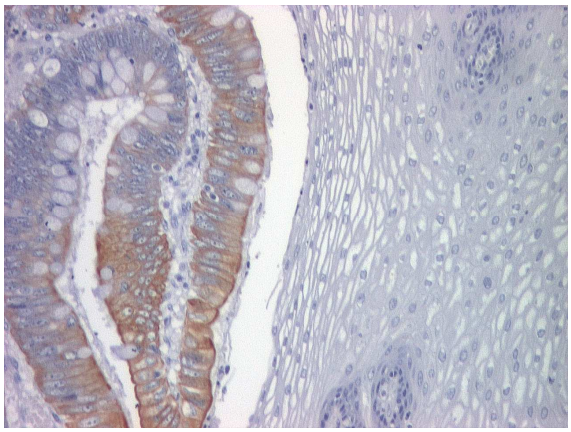

Figure 6. *Weak CK20 immunostaining of the superficial epithelium, (ob. 10x)*

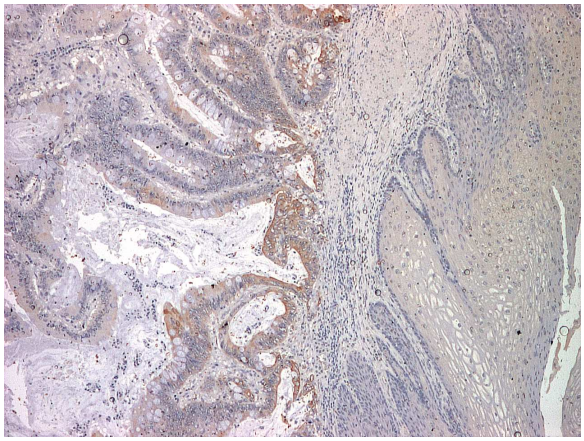

Figure 7. *Weak CK 7 immunostaining of both epithelium, (ob. 4x)*

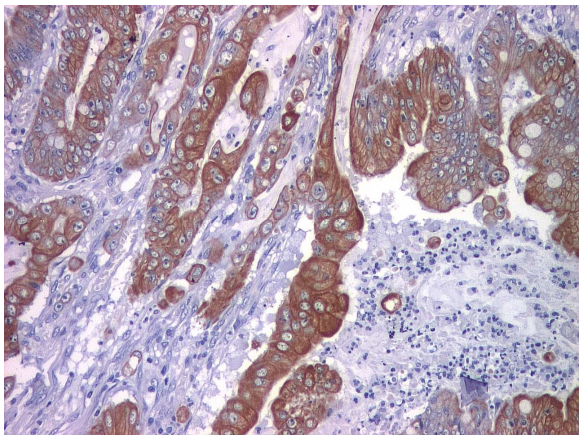

Figure 8. *Strong CK 20 diffuse immunostaining, (ob. 10x)*

These results showed that although there was a significant difference in CK 7/20 immunoreactivity pattern between esophageal or gastric metaplasia and adenocarcinoma, there were also several cases which did not belong to either Barrett's or gastric pattern.

Relationship between CK7/20 immunoreactivity pattern and clinical findings

Comparing the differences between the expression of CK7/20 immunoreactivity pattern and the positive prevalence of clinical or endoscopic findings at different sites of metaplasia and adenocarcinoma, we observed that the positive prevalence of gastro-esophageal reflux symptoms in the cases with Barrett's CK7/20 pattern was significantly higher than those with gastric CK7/20 pattern. Not significant difference were found for other findings: age, sex, weight, smoking history and alcohol consumption.

Comparing endoscopic findings, patients with Barrett's CK7/20 pattern were more likely to have a normal appearance of the stomach whereas those with the gastric CK7/20 pattern were more likely to have a erythematous or atrophic appearance.

Comparing *Helicobacter pylori* infection data, the positive prevalence of *H. pylori* infection in the cases with gastric CK7/20 pattern was significantly higher than those with Barrett's CK7/20 pattern.

4. Discussion

We investigated several biological characteristics of patients with Barrett's metaplasia or cardiac intestinal metaplasia because the distinction between these two entities is important, since the etiology and risk of developing adenocarcinoma are different [9,10]. The best criterion for the distinction between adenocarcinomas is the precise knowledge that the biopsy specimens are from the esophagus or the stomach, but because of the effects of the anatomic variation of gastro-esophageal junction and squamocolumnar junction, inflammation and hiatus hernia, this is not always possible in endoscopy. In histological morphology there is also no significant difference between Barrett's metaplasia and cardiac intestinal metaplasia. Therefore, we think that the immunoreactivity for CK7 and CK20 could be used to distinguish Barrett's metaplasia from cardiac intestinal metaplasia.

The study of cytokeratins CK7/20 immunoreactivity are shown: the "Barrett's CK7/20 pattern" was found in Barrett's metaplasia and esophagus adenocarcinoma, in agreement with other studies [11-14] on the contrary, the preponderant CK7/20 immunoreactivity pattern in cardiac intestinal metaplasia and gastric cardia adenocarcinoma was "gastric CK7/20 pattern", but there were also several cases which did not belong to either Barrett's or gastric pattern.

In our study, we found that the prevalence of Barrett's CK7/20 pattern in Barrett's metaplasia was similar to esophagus adenocarcinoma, but significantly higher in the esophagus compared to cardiac metaplasia or adenocarcinoma. On the other hand, the results indicated that the prevalence of gastric CK7/CK20 pattern was significantly higher in cases with gastric cardiac metaplasia or adenocarcinoma.

These results implied that CK7/20 immunoreactivity pattern could be used to distinguish most of esophagus adenocarcinoma from gastric cardiac adenocarcinoma. In our study, there were a number of cases which belonged to neither Barrett's or gastric pattern and could not distinguish all cases of esophagus adenocarcinoma from gastric cardiac adenocarcinoma.

We identified two subgroups of patients: the first subgroup (group I and III) had a Barrett's CK7/CK20 pattern (11 of 17 cases) and features of Barrett's metaplasia, including gastro-esophageal reflux symptoms, normal gastric appearance and a low frequency of *Helicobacter pylori* infection.

Despite preponderance for male patients in this subgroup, the sex difference was not significant.

In contrast, the second subgroup (group II and IV) had a gastric CK7/CK20 pattern (10 of 16 cases) and features including antral chronic gastritis with atrophy and intestinal metaplasia, and high frequency of *Helicobacter pylori* infection.

These results indicate that the CK7/20 immunoreactivity pattern combined with gastro-esophageal reflux symptoms and statuses of Helicobacter pylori infection could imply the probable cause of esophagus adenocarcinoma or gastric cardia adenocarcinoma and help to distinguish from each other.

5. Conclusions

We conclude that the distinction of esophageal adenocarcinoma from gastric cardia adenocarcinoma should not be based on a single method but the combination of clinical characteristics, histological results, CK7/20 immunoreactivity and especially precise endoscopic biopsy.

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